

## 9.

Sex Determination in *Platypoecilus maculatus*.

## II. History of a Male Platyfish that Sired All-female Broods.

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(Plates I-IV; Text-figure 1).

The cytological and genetical aspects of sex determination have been emphasized by Bridges (1939), the physiological influences by Goldschmidt (1938), and the developmental, endocrine and environmental factors by Willier (1939), Danforth (1939) and Witschi (1939). The consensus is that the sex of most vertebrate animals is the product of the interaction of many endogenous and exogenous forces. In some animals, however, the genetic or chromosomal mechanism for sex determination has attained greater influence than the exogenous agents. For example, the genetic factors are stabilized in the teleost fishes *Platypoecilus*, *Lebistes* and *Oryzias*, as in *Drosophila*. Knowledge of sex-linked inheritance in these forms makes the results of genetic experiments predictable. When exceptions to expectancy appear, they tend to strengthen rather than to weaken the basic principles. For example, in the platyfish, *Platypoecilus maculatus*, Gordon (1946a) found an exceptional male which had a phenotype usually associated with that of a female. The unexpected male was tested with a normal female platyfish and proved to be a functional male. Gordon (1947) suggested that the exceptional male was a product of genetic sex reversal because it retained the chromosomal constitution of a female. This was revealed when the exceptional male and the normal female produced a series of broods which totalled 153 young. All were female.

## GENETIC ANALYSIS OF THE SEX-REVERSED PLATYFISH.

The female parent of the exceptional male platyfish was heterozygous for the striped side (*Sr*) gene. Its male parent carried the spotted dorsal fin (*Sd*) gene on its Y chromosome and a spotted side (*Sp*) gene on its X. These genes are but three of a series of five dominant, sex-linked alleles for the develop-

ment of macromelanophore patterns, which are described in detail by Gordon (1948).

The genetic data were represented by Gordon (1947) essentially as follows:

	P <sub>1</sub>	
Striped Female		Spot-sided, Spotted dorsal fin Male
(X) <i>Sr</i> / (X) +		(X) <i>Sp</i> / (Y) <i>Sd</i>
	F <sub>1</sub>	
Daughters		Sons
32 (X) <i>Sr</i> / (X) <i>Sp</i> , striped and spotted		29 (X) <i>Sr</i> / (Y) <i>Sd</i> , striped, spotted dorsal
34 (X) <i>Sp</i> / (X) +, spotted		27 (X) + / (Y) <i>Sd</i> , spotted dorsal
		1 (X) <i>Sp</i> / (X) +, spotted, <i>exceptional type</i>

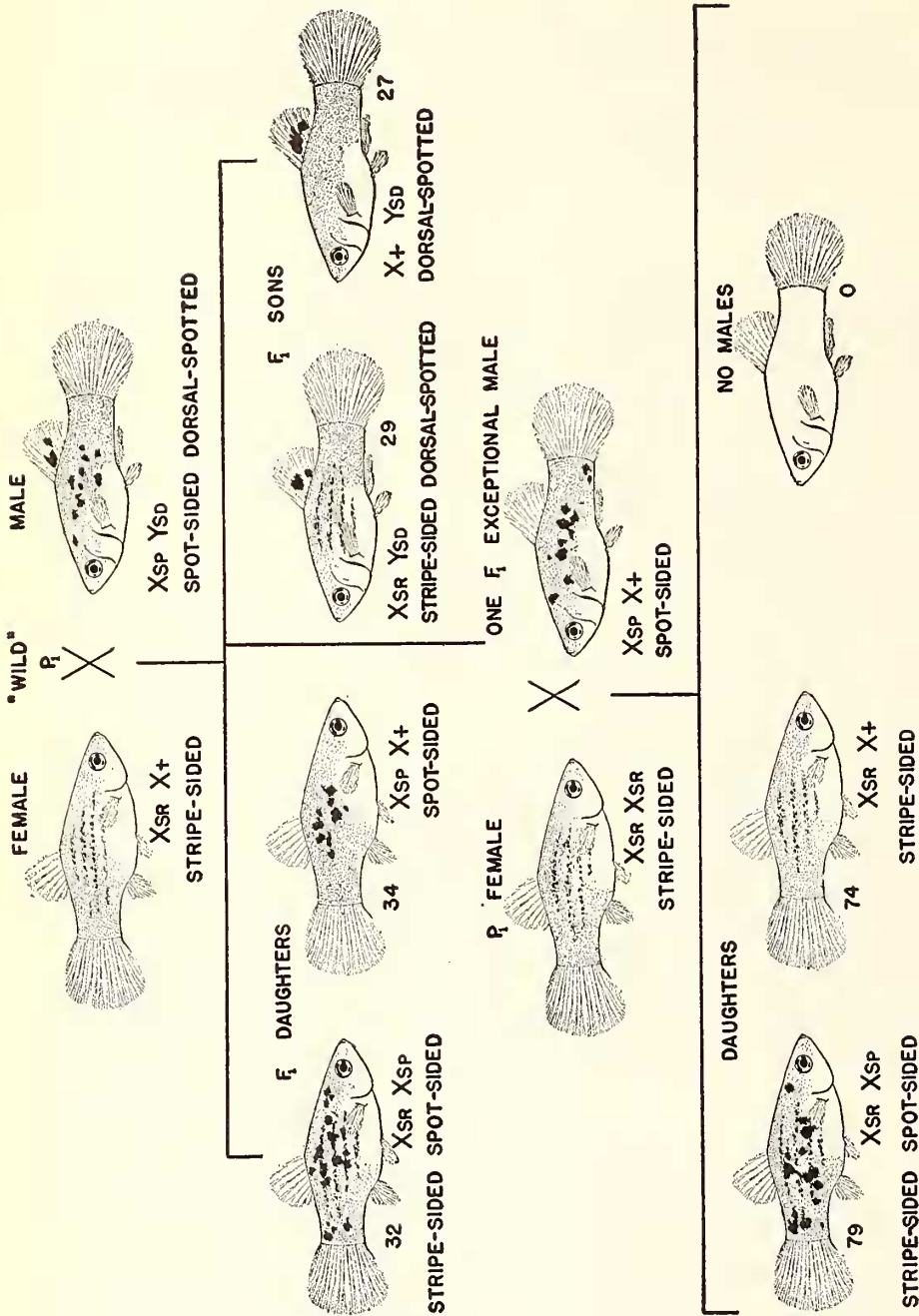
All but one of the 35 spot-sided animals, (X) *Sp* / (X) +, were females. The one spotted son was either the product of a crossing-over of the sex chromosomes in its male parent (X) *Sp* / (Y) *Sd*, which produced a (Y) *Sp* gamete and an (X) + / (Y) *Sp* individual, or it was the result of sex reversal of an (X) + / (X) *Sp* female.

To test which of these two possibilities was correct, the exceptional spotted male was mated to a homozygous stripe-sided female, (X) *Sr* / (X) *Sr* (Pl. I, Fig. 1). If the abnormal male resulted from a cross-over, and was (X) + / (Y) *Sp*, 50% of his offspring would be female and 50% would be male, and all the males would be spotted. If he had the genetic constitution of a female, (X) *Sp* / (X) +, all of his offspring would be female, and one-half of the females would be spotted.

The exceptional spotted male, mated with a normal, homozygous striped female, produced 153 offspring, all of which were female; 79 were spotted and striped and 74 were merely striped (Pl. I, Fig. 2). These data suggest that the exceptional spotted male was the product of sex reversal and that it had retained the unaltered chromosomal constitution of a female. This may be expressed genetically as follows (Text-fig. 1):

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PLATYPOECILUS MACULATUS



TEXT-FIG. 1. The mating which led to the discovery of the functional male having the genetic constitution of a female. A striped-sided female, XX, (63-1), was mated to a spot-sided, spotted dorsal male, XY, (62-11), both of which were of the Rio Jamapa, Mexican stock. In their F<sub>1</sub> population only spot-sided females were expected, but a single male of this phenotype appeared. When the exceptional spot-sided male was tested by mating it to a homozygous stripe-sided female, XX, the parents produced 153 daughters but no sons. From this and other evidence it was concluded that the genetic constitution of the exceptional spot-sided male (150-91) was XY like that of normal females, rather

	P <sub>1</sub>	
Female		Male (exceptional)
(X)Sr/(X)Sr		(X)Sp/(X)+
	F <sub>1</sub>	
Females		Males
79 (X)Sr/(X)Sp		None
74 (X)Sr/(X)+		None

The spotted male sired the total of 153 daughters in three separate broods which were born at monthly intervals. The three broods could have been produced by a single insemination because platyfish females have the means for storing viable sperm within the folds of their oviducts for four to five consecutive broods. Although the male was in the company of the female constantly for five months, and for two months after the last brood, no more than three broods were produced.

The same female, (X)Sr/(X)Sr, was subsequently isolated for two additional months during which period she did not produce any young. She was then mated to a second, but unspotted, male, (X)+/(Y)+, and from this second mating she produced stripe-sided male and female offspring.

It was concluded, therefore, (1), that the test female was not sterile and that its sex-determining mechanism was (X)Sr/(X)Sr, and (2), that the exceptional spotted male, (X)Sp/(X)+, was essentially responsible for the unusual production of the all-female brood of the 153 offspring.

#### HISTOLOGY OF THE GONAD IN THE SEX-REVERSED MALE.

The sex-reversed male, after it appeared to be sterile, was fixed in Bouin's fluid. Its testis was dissected, sectioned, stained and examined histologically. For comparative study, testes were also examined from young adult fertile males and from older senile males that had previously sired many broods but had passed their active reproductive period.

The histological picture of the testis in the normal platyfish reveals that it is a single, fused, acinar gland in which the spermatogonial elements are organized into globular units rather than into seminiferous tubules (Pl. II, Fig. 3). The primary spermatogonia are at the periphery of the gland, and from the periphery to the center there are closely packed acini containing primary spermatocytes, secondary spermatocytes, spermatids and spermatophores. The spermatophores are spherical masses of mature spermatozoa the heads of which lie at the periphery, embedded in Sertoli cells, and the tails in the center of the sphere. (During copulation, the spermatophores are transferred by means of the male's modified anal fin or gonopodium to the urogenital orifice of the female. Within the female, the spermatophores break up and the spermatozoa are free).

The center of the testis has two main branching sperm ducts which are usually

filled with spermatophores. When the ducts are filled with spermatophores their walls are somewhat distended, and at this time their epithelial cells are cuboidal; at other times they are columnar. Some interstitial tissue is found around the ducts and a bit of it between the acini. The ducts contain a small trace of nongranular "colloidal" material which takes a faint acidophilic stain. The composition and function of the colloid are unknown.

The testes from two old swordtail males that had been fertile but were no longer capable of inseminating females were studied for comparative purposes. The swordtails were used because they were available. The histological structures of the testes of adult platyfish and swordtails are practically identical.

The testis of one old swordtail has definite signs of disorganization and disintegration (Pl. II, Fig. 4). The spermatogonial elements are scattered irregularly throughout the gland. The non-glandular, connective, interstitial tissue, containing many degenerating sperm, is abundant. The ducts contain spermatophores, many of which are in the process of disintegration. The ducts contain much colloid.

Another testis taken from an old swordtail was studied. The male had been kept with a female for many months, during which time no young were produced. When, however, the female was autopsied she was gravid. The gonad of the second male had similar abnormalities to the first, but the irregularities were not so pronounced.

A cross-section of the testis of the sex-reversed platyfish reveals a highly abnormal gland (Pl. III, Fig. 6; Pl. IV, Fig. 7). The most noticeable defect is the great enlargement of the sperm ducts, the walls of which are composed of squamous cells. The distended ducts, which occupy most of the testis, contain much colloid, spermatophores in the process of disintegration, free spermatozoa and a few normal spermatophores. The gland is almost devoid of sex cells in their early stages of spermatogenesis.

At the periphery of the testis there are a few nests of primary germ cells. Toward the center between the branches of the sperm ducts there are large masses of degenerating primary germ cells. Plate IV, Fig. 8, shows a series of germ cells; those at the far right are normal, and those at the far left, in the process of disintegration. In this connection, Wolf (1931) describes degenerating germ cells in all stages in the development of the testis except the last, in the mature gland.

The interstitial tissue in the testis of the sex-reversed platyfish seems normal. No degenerate sperm are found outside of the sperm ducts. The organization of the gland is unusual in that there are relatively few early spermatogonial elements, but those that are present are in their normal position. These anomalies are not comparable to those of the testes of the aged swordtail males.

The gonopodium of the sex-reversed male platyfish was normal.

#### DISCUSSION.

Sex reversal in a male platyfish was reported by Breider (1942) prior to the one Gordon (1947) discovered, but Breider did not describe the histology of the exceptional male's gonad. Tavalga (1949), in the course of an endocrinological analysis, found a young platyfish that had the phenotypic, sex-linked color pattern of a female but a typical juvenile male gonad, but this was not analyzed genetically.

The sex-reversed male platyfish described in this paper is of particular interest because its origin and genetic history are known. After the platyfish was fixed for histological analysis its gonopodium was examined and found to be normal; this showed that its incapacity to continue to function as a fertile male could not be attributed to a disfunction in this important secondary sex character.

The sex-reversed male platyfish had inseminated a female successfully. It therefore had a normal, or at least a functional, gonad for a time. The eventual disfunction of the testis was not due to the premature senility of the male because histologically its gonad was totally different from the involuted gonads characteristic of senescent males. The sex-reversed male was only 14 months old and therefore was not senile at the time it was fixed.

The gonad of the sex-reversed male may have become abnormal owing to an upset in the hormonal balance in the organism as a whole. All the histological abnormalities reported in the sex-reversed platyfish are found in studies of testes of *Lebistes* which are experimentally treated with hormones. For example, in one of his figures Berkowitz (1941) illustrated the presence of free spermatozoa in the sperm ducts of sterile guppy males that had previously received injections of estrogens. Furthermore, Eversole (1941) reported a scarcity of early spermatogonial stages in the testes of guppies that had been treated with pregneninolone, a hormone that has a powerful androgenic effect in fishes.

Aronowitz & Gordon (Ms.) also detected spermatophore disintegration and a scarcity of early spermatogonial stages in hybrids between the platyfish (*P. maculatus*) and swordtail (*X. hellerii*) (Pl. III, Fig. 5). Gordon & Rosen (1951) suggested that the abnormal, nonfunctional testes in some hybrids may have been produced by the endocrine imbalance initiated by the union of dissimilar sex chromosomes. Friess (1933) found free sperm in the sperm ducts of some sex-reversed swordtail males. She suggested that this was indicative of sex-reversed fishes. In the sex-reversed platyfish male we found no evidence, at the time it was examined, of any ovarian elements, but the XX platyfish may have had an incipient ovary quite early in life.

Cohen (1946), after subjecting male

platyfish at two weeks of age to the effects of alpha-estradiol benzoate for twenty weeks, found that their testes were small, bipartite and compact. The sperm duct epithelium was columnar, the interstitial tissue was profuse, no ova were present, spermatophores and spermatids were few. Equally young males treated for shorter periods, for eight to twelve weeks, showed ova in ova-testes, but males treated for twenty weeks showed a falling off of the inhibiting effects of the estrogen. Cohen also studied the effect of pregneninolone on young, genetically determined female platyfish. The ovaries of treated fish were much smaller than those of their controls; mature ova with their usual complement of yolk were entirely wanting. Tavalga (1949) repeated some of this work and obtained similar results.

From the data presented, an interpretation of the process of sex reversal in the exceptional (X)Sp/(X) + male may be outlined. Some hormonal imbalance (induced probably by a fortuitous combination of genes for sex determination carried on both sex and autosomal chromosomes) transformed a potential female platyfish to a functional male. To use Danforth's terms, its genetic sex was female, its real sex was male.

In the process of sex reversal, which is interpreted as having been aberrant from start to finish, a stage was reached in which the exceptional, genetically XX platyfish with all the organs of a normal, functional male was capable of copulation and successful insemination. The duration of this stage of sexual normality and fertility was brief. The hormonal imbalance inherent in the XX male continued, and eventually caused the testis to become abnormal. Subsequently, the testis was incapable of providing functional spermatozoa. As a consequence the male was, in effect, sterile. Its sexual incapacity was reached far in advance of the ordinary period of senescence.

Investigators studying embryonic differentiation and development in vertebrate animals have been impressed with the striking effects of sex hormones on fishes. Witschi (1942) attributes this in part to the peculiarities of their genetical constitution, saying that the lower vertebrates have no completely developed genetical mechanism of sex determination. Consequently, he says, they are, at least during early development stages, in a relatively labile condition and secondary influences can comparatively easily shift sex determination in one direction or the other. He adds that the situation is complicated by the fact that embryologically as well as genetically the species studied are not completely enough analyzed.

The general opinion that fishes have a labile mechanism for sex determination has been derived, in some measure, from study of the swordtail, *Xiphophorus hellerii*. It arose from the widely cited early work of Essenberg (1926), who showed that functional sex reversal occurs spontaneously in

this species. It is a rare phenomenon. It may have been forgotten that Essenberg had only two swordtails which had first been functional females and which later became functional males. Several workers after Essenberg showed that some swordtails are protogynous. Some of the young pass through a female-like stage of gonadal development before becoming functional males. After Essenberg, no new instances of complete and functional sex reversal in this species have been reported. From the numerous citations referring to the original sex-reversed swordtail one gets the erroneous impression that the phenomenon is common.

On the genetic level, the swordtail is a puzzling species with reference to its mechanism for sex determination. Suggestions made to explain the mechanism are contradictory. In the main this is because no sex-linked genes have, as yet, been discovered in this species. In this connection, Witschi (1939), basing a statement on Breider (1936), said that the heterochromosome of *Platypoecilus maculatus* is homologous with one in the closely related *Xiphophorus hellerii*. Breider's evidence is somewhat ambiguous. Breider thought that the gene *Mo*, for montezuma pattern, was an attribute of the swordtail. He then pointed out that *Mo* was one of a series of sex-linked alleles which also contained a number of platyfish genes, *N*, *Sp*, etc. Gordon (1946b, 1948) showed that the so-called *Mo* gene of the swordtail is actually a combination of two platyfish genes, *Sr* and *Dr*. These platyfish genes, according to Gordon's interpretation, had been incorporated into swordtail germ plasm through a process of introgressive hybridization. The transfer of genes was accomplished by fish fanciers' breeding methods under conditions of domestication.

The term heterochromosome with reference to the sex chromosomes of *Platypoecilus maculatus* is unsatisfactory because the platyfish has a duality of sex-determining mechanisms. In natural populations of this species from three geographically isolated rivers in Mexico, the genetic mechanism for sex determination is XX for females and XY for males. In a natural population from the Belize River in British Honduras, and in domesticated stocks, such as Breider and other geneticists had previously studied (see Gordon, 1947), the genetic mechanism for sex determination is WY (or WZ) for females and YY (or ZZ) for males. By "tagging" the platyfish sex chromosomes with sex-linked genes, and then hybridizing them with the swordtail, some information was obtained on the compatibility of each kind of platyfish sex chromosome with the pairing member chromosome from the swordtail in the platyfish-swordtail hybrids. Some of the interesting results obtained were described by Gordon (1948) and their importance as isolating mechanisms are outlined by Gordon & Rosen (1951).

No generalized statement on the strength or weakness of the genetic mechanisms for sex determination in fishes is satisfactory at this time. In *Platypoecilus maculatus* and in *P. variatus* and *P. xiphidium*, as well as in *Lebistes* and in *Oryzias*, they are definitely stable despite some exceptions. In other species of teleosts which have been studied, for example in *Xiphophorus hellerii*, and in *X. montezumae*, as well as in two of the three species of *Limia*, and in *Macropodus*, *Carpio* and *Carassius*, the mechanism is unknown.

#### SUMMARY.

1. The origin and genetic background of a platyfish, *Platypoecilus maculatus*, with the genetic sex chromosome mechanism of a female and the morphology and physiology of a male, is described. It was found in a stock in which the males are heterogametic (XY) and the females homogametic (XX). When the exceptional XX male was mated to a normal XX female, 153 offspring were produced, all of which were XX females.

2. The XX sex-reversed male was fertile for a brief period only. When it was sterile it was fixed and sectioned. A histological examination of its testis revealed hypertrophied sperm ducts, spermatozoa-free outside of spermatophores, early spermatogonial stages scarce and masses of degenerating primary germ cells. These features denote sterility. Similar histological conditions have been reported in the testes of related fishes after treatment with sex hormones. It has been suggested that the premature sterility in the sex-reversed male was due to a hormonal imbalance, brought about by androgenic agents acting upon a genetically constituted female.

#### POSTSCRIPT.

While the present paper was in press a paper by Albert W. Bellamy and Marion L. Queal, entitled "Heterosomal inheritance and sex determination in *Platypoecilus maculatus*," was published in *Genetics*, 36(1): 93-107, 1951. The authors described a series of matings of domesticated (YY-YW) platyfish which produced 10,686 offspring; 5,324 of them were male, 24 of which were exceptional with reference to their phenotypes. Most of the exceptional males were tested in another series of matings. Seven of them apparently were genetic sex-reversals.

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## EXPLANATION OF THE PLATES.

## PLATE I.

- Fig. 1. The male with the genetic constitution of a female *Platypoecilus maculatus* (Pedigree 159-21) is shown at the lower left. It carries the sex-linked gene *Sp* for macromelanophores on the sides and the autosomal gene, *Co*, for comet, a faint line along the upper and lower margin of the tail. The standard platyfish female is at the right. It carries the sex-linked gene *Sr*, for striped sides and two autosomal genes, *Cc*, for complete-crescent, and *O*, for one-spot. Note the dimorphism in total size, shape, and specifically in the structure of the anal fin. This pair produced 153 offspring all of which were female. See figure 2.
- Fig. 2. The females produced by the pair shown in Fig. 1 were of two types with reference to macromelanophore genes. About half of them were spot-sided, gene *Sp*; one is shown at the bottom of the figure. The other half were recessive for this gene, +; one is shown at the top. Note that the tail patterns are the recombination products of the parental types. The non-spot-sided female, the upper, has the *OCo* genes for one-spot and comet while the spot-sided one, the lower, has *Cc*, the gene for complete-crescent. Every female tested proved to be fertile (Pedigree 167).

(Photographs of Figures 1 and 2 made by S. C. Dunton, Staff Photographer, New York Zoological Society).

## PLATE II.

- Fig. 3. Longitudinal section of the testis of a young, normal, sexually mature platyfish male, cut at 7 micra and stained with hematoxylin and eosin. Magnification approx. 100X. The arrangement of the spermatogonial elements is characteristic; the acini which contain the early stages of spermatogenesis are near the periphery of the gland, and the acini which contain the later stages of spermatogenesis are near the center. The sperm duct is filled with closely packed spermatophores. **D**, sperm duct;

**SC**, spermatocyte acinus; **SP**, spermatophore acinus; **ST**, spermatid acinus.

- Fig. 4. Cross-section of a testis of a senescent swordtail male, cut at 7 micra and stained with Masson's trichrome stain. Magnification approx. 100X. The arrangement of the spermatogonial elements is not orderly. The number of acini is small. Large amounts of stromal tissue are present. Degenerating sperms are found both within and outside of the sperm ducts. **D**, sperm duct; **DS**, degenerating sperms; **S**, stroma.

## PLATE III.

- Fig. 5. Cross-section of the testis of a platyfish-swordtail hybrid, cut at 7 micra and stained with hematoxylin and eosin. Magnification approx. 100X. The sperm ducts fill most of the testis and contain free sperm. This gland resembles that of the sex-reversed male. **D**, sperm duct; **FS**, free sperm.
- Fig. 6. Cross-section of the testis of the sex-reversed male, cut at 7 micra and stained with Masson's trichrome stain. Magnification approx. 130X. The sperm ducts occupy most of the testis and they contain many free sperm. The number of germ cell acini is small. **DGC**, group of degenerating germ cells; **FS**, free sperm.

## PLATE IV.

- Fig. 7. Free sperms in the sperm duct of the sex-reversed male, cut at 7 micra and stained with Masson's trichrome stain. Magnification approx. 1,800X. One spermatophore is in a stage of degeneration. A number of free sperm are suspended in colloid. **FS**, free sperm; **SP**, spermatophore.
- Fig. 8. Primary germ cells in the testis of the sex-reversed male, cut at 7 micra and stained with Masson's trichrome stain. Magnification approx. 1,000X. Those at the right are normal and take acid fuchsin, those at the left are degenerate and take aniline blue. **DGC**, degenerating primary germ cell; **PGC**, normal primary germ cell.